

## Problem 1

I confirmed that my network was balanced through the use of `checkAllBalances()`, a function which goes through all reactions in the network and confirms that an equal number of atoms of each species are found as reactants and products. Reactions where a species disappears (exchange fluxes) are not balanced, and neither is the biomass production reaction, as biomass is a fictitious species, used to quantify cell growth. Through the use of dynamic flux analysis and the bounds given on oxygen uptake, glucose uptake, and growth rate, I was able to predict with a high degree of accuracy the rate at which *E. coli* would uptake glucose, as shown in Figure 1. By setting the maximum oxygen uptake rate to zero, I was able to predict the anaerobic fluxes, as shown in Figure 2. I used an initial biomass concentration of .001 g/L, since this gave a good fit to the glucose uptake and biomass curve, since no initial value was given by Palsson. Initial concentrations of all species other than glucose and acetate were assumed to be zero, and initial concentrations of glucose and acetate were set based on the data in figure 7 of [1].

To perform dynamic flux balance analysis (dFBA), I solved

$$\frac{dX}{dt} = Sv \quad (1)$$

where  $X$  gives the species concentrations as a function of time,  $S$  is the stoichiometric matrix, and  $v$  represents the fluxes through the network, as determined by FBA. This equation neglects the growth and dilution terms in a complete mass balance, as they are small. I solved the differential equation using Euler's method with a time step of .1 hours. For the purposes of FBA, I formulated my objective as maximizing the flux through the biomass producing reaction.

There are two ways in which *E. coli* can generate ATP-through substrate level phosphorylation and oxidative phosphorylation. Substrate level phosphorylation generates ATP by reactions between organic molecules, whereas oxidative phosphorylation uses ATP synthase with a proton gradient to produce ATP [2]. When *E. coli* uptake glucose, they invest two ATP convert it to pyruvate, though either glycolysis, pentose phosphate, or Entner Doudoroff pathways. Going through glycolysis increases the amount of ATP available, as it produces 2 ATP for each glucose molecule processed. If oxygen is not present, this pyruvate is used for fermentation, which uses substrate level phosphorylation to produce ATP. While fermentation is faster than oxidative phosphorylation, it is not as energetically efficient [2]. When oxygen is available, the pyruvate generated through glycolysis can continue through the citric acid cycle, which produces NADH that carries electrons the electron transport chain (which requires an electron acceptor, like oxygen to run), which produces the proton gradient necessary to run ATP synthase [3].

If we examine the flux profiles in the aerobic and anaerobic environments, without applying the bounds given by Palsson's experimental data, we observe that glucose uptake rates are the same, but the flux through biomass production is much larger in the aerobic case, suggesting that energetic species are available for non-essential reactions, like growth. `R_atp`, which represents the creation of ATP by ATP has no flux through it in the anaerobic case, as expected, and a fairly large flux through it in the presence of oxygen. Instead, there is a higher flux through reactions that produce ATP through substrate level phosphorylation such as `R_pgk` and `R_pyk`. Furthermore, in the anaerobic case, ethanol and formate are exported from the cell, which is not observed in the aerobic case. The complete flux profiles for both cases are given in the supplemental material.

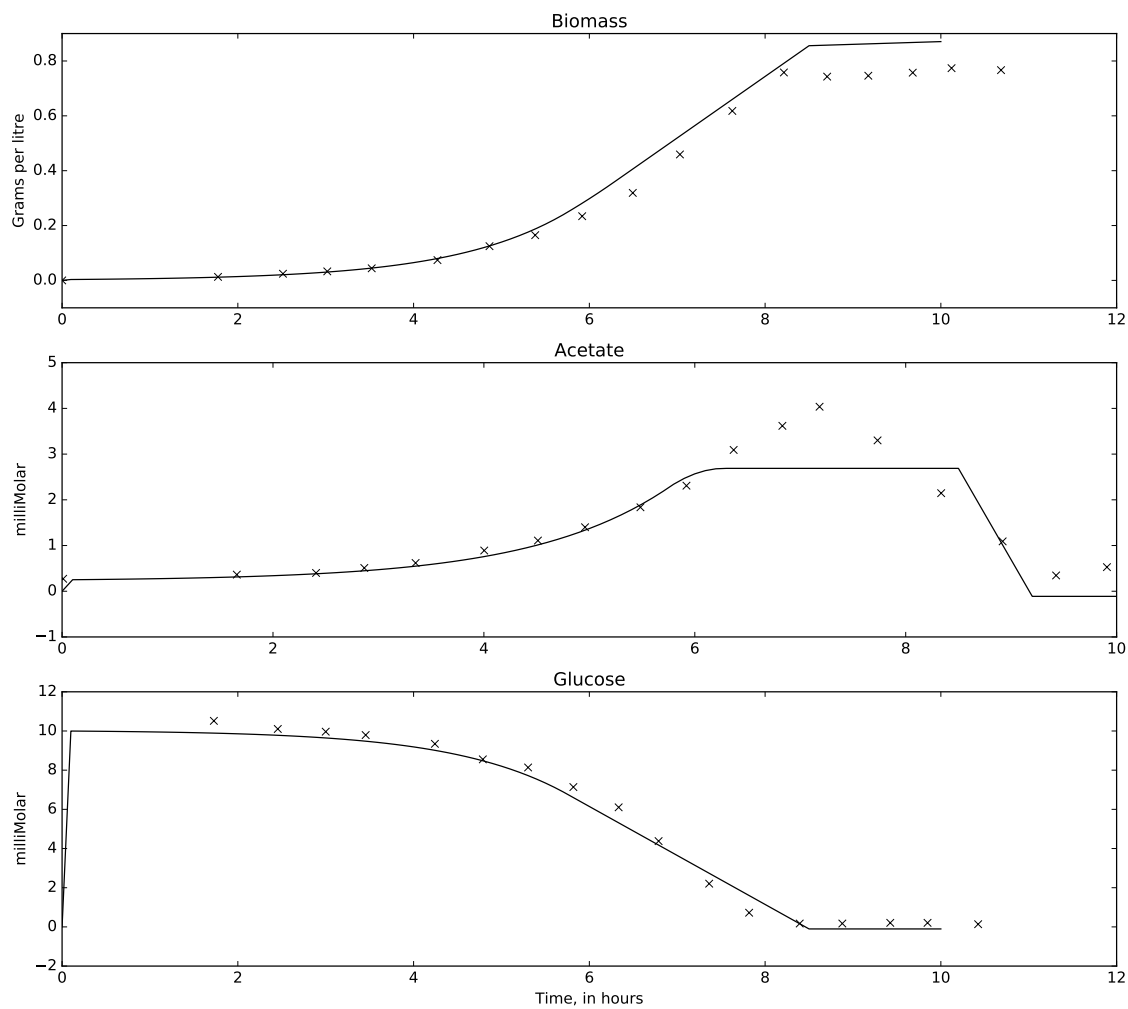


Figure 1: Concentrations of selected metabolites as a function of time as calculated by dFBA, grown in a batch culture in the pretense of oxygen. Lines show predicted data, xs show experimental data extracted from [1].

## References

- [1] A. Varma and B. O. Palsson, "Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type escherichia coli w3110.," *Applied and environmental microbiology*, vol. 60, no. 10, pp. 3724–3731, 1994.
- [2] T. Conway, P. S. Cohen, *et al.*, *Metabolism and bacterial pathogenesis*. American Society for Microbiology (ASM), 2015.
- [3] B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*. Garland Science, 2014.

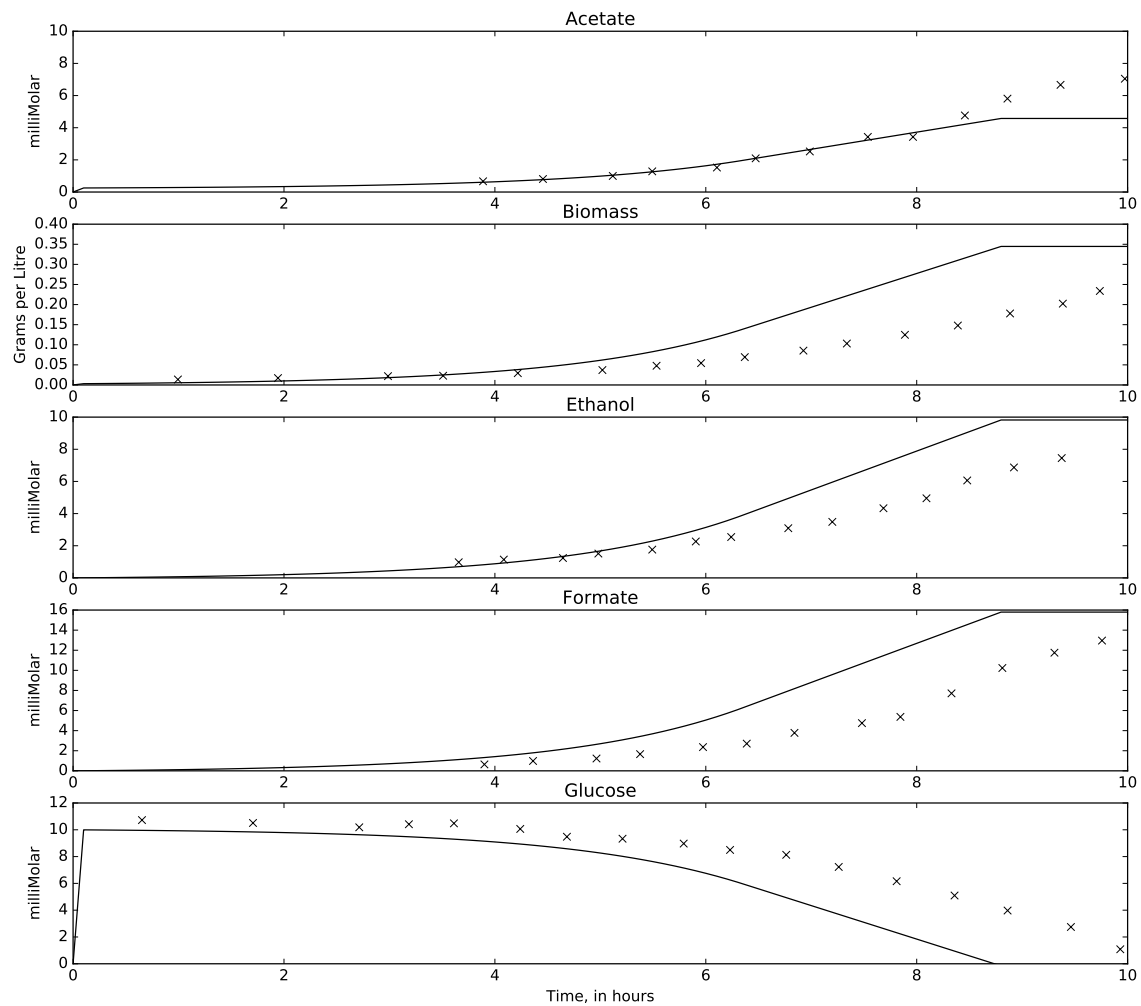


Figure 2: Concentrations of selected metabolites as a function of time as calculated by dFBA, grown in a batch culture in the absence of oxygen. Lines show predicted data, xs show experimental data extracted from [1].

# Supplemental Material

## Steady State Fluxes in Aerobic Environment

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1,R_glk_hex::M_pep_c+M_glc_D_e --> M_pyr_c+M_g6p_c,2.5
3,R_pgi::M_g6p_c --> M_f6p_c,1.1041123916076936
5,R_pfk::M_atp_c+M_f6p_c --> M_adp_c+M_fdp_c,1.8270632175924555
7,R_fbaA::M_fdp_c --> M_dhap_c+M_g3p_c,1.8270632175924555
9,R_tpiA::M_dhap_c --> M_g3p_c,1.8270632175924555
11,R_gapA::M_g3p_c+M_nad_c+M_pi_c --> M_13dpg_c+M_h_c+M_nadh_c,3.9541747758961754
13,R_pgk::M_13dpg_c+M_adp_c --> M_3pg_c+M_atp_c,3.9541747758961754
15,R_gpm::M_3pg_c --> M_2pg_c,3.6188531187805015
17,R_eno::M_2pg_c --> M_h2o_c+M_pep_c,3.6188531187805015
19,R_pyk::M_adp_c+M_pep_c --> M_atp_c+M_pyr_c,0.3601878695562891
21,R_ppc::M_co2_c+M_h2o_c+M_pep_c --> M_oaa_c+M_pi_c,0.6423113239509861
22,R_pdh::M_coa_c+M_nad_c+M_pyr_c --> M_accoa_c+M_co2_c+M_nadh_c+M_h_c,2.2252285177666624
24,R_zwf::M_g6p_c+M_nadp_c --> M_6pgl_c+M_h_c+M_nadph_c,1.3499377823838083
26,R_pgl::M_6pgl_c+M_h2o_c --> M_6pgc_c,1.3499377823838083
27,R_gnd::M_6pgc_c+M_nadp_c --> M_co2_c+M_nadph_c+M_ru5p_D_c+M_h_c,1.3499377823838081
28,R_rpe::M_ru5p_D_c --> M_xu5p_D_c,0.7388427414189204
31,R_rpi_reverse::M_ru5p_D_c --> M_r5p_c,0.6110950409648879
32,R_talAB::M_g3p_c+M_s7p_c --> M_e4p_c+M_f6p_c,0.409879632146211
34,R_tkt1::M_r5p_c+M_xu5p_D_c --> M_g3p_c+M_s7p_c,0.409879632146211
36,R_tkt2::M_e4p_c+M_xu5p_D_c --> M_f6p_c+M_g3p_c,0.32896310927270944
40,R_gltA::M_accoa_c+M_h2o_c+M_oaa_c --> M_cit_c+M_coa_c,1.3851760401342272
41,R_acn::M_cit_c --> M_icit_c,1.3851760401342272
43,R_icd::M_icit_c+M_nadp_c --> M_akg_c+M_co2_c+M_nadph_c+M_h_c,1.3851760401342272
45,R_sucAB::M_akg_c+M_coa_c+M_nad_c --> M_co2_c+M_nadh_c+M_succoa_c+M_h_c,1.1433454680338926
46,R_sucCD::M_adp_c+M_pi_c+M_succoa_c --> M_atp_c+M_coa_c+M_succ_c,1.1433454680338928
47,R_sdh::M_q8_c+M_succ_c --> M_fum_c+M_q8h2_c,1.1433454680338928
49,R_fum::M_fum_c+M_h2o_c --> M_mal_L_c,1.1433454680338928
51,R_mdh::M_mal_L_c+M_nad_c --> M_oaa_c+M_h_c+M_nadh_c,1.1433454680338928
53,R_gdhA::M_akg_c+M_nadph_c+M_nh3_c+M_h_c --> M_glu_L_c+M_h2o_c+M_nadp_c,1.1649065402378806
55,R_glnA::M_glu_L_c+M_atp_c+M_nh3_c --> M_gln_L_c+M_adp_c+M_pi_c,0.05731400248962422
58,R_cyd::2.0*M_h_c+0.5*M_o2_c+M_q8h2_c --> M_h2o_c+M_q8_c+2.0*M_he_c,10.404483760457897
60,R_atp::M_adp_c+M_pi_c+4.0*M_he_c --> M_atp_c+4.0*M_h_c+M_h2o_c,9.832811026440949
61,R_nuo::3.0*M_h_c+M_nadh_c+M_q8_c --> M_nad_c+M_q8h2_c+2.0*M_he_c,9.261138292424002
83,R_Biomass_Ecoli_core_w_GAM::1.496*M_3pg_c+3.7478*M_accoa_c+59.81*M_atp_c+0.361*M_e4p_c+0.0709*M_f6p_c
+0.129*M_g3p_c+0.205*M_g6p_c+0.2557*M_gln_L_c+4.9414*M_glu_L_c+59.81*M_h2o_c+3.547*M_nad_c+13.0279*M_nadph_c+1.7867*M_oaa_c
+0.5191*M_pep_c+2.8328*M_pyr_c+0.8977*M_r5p_c -->
59.81*M_adp_c+4.1182*M_akg_c+3.7478*M_coa_c+59.81*M_h_c+3.547*M_nadh_c+13.0279*M_nadp_c+59.81*M_pi_c+M_bio_c,0.224145492724381
84,M_o2_c_exchange::M_o2_e --> M_o2_c,5.202241880228948

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85,M\_co2\_c\_exchange::M\_co2\_c --> M\_co2\_e,5.461376484367605  
 86,M\_h\_c\_exchange::M\_h\_c --> M\_h\_e,15.53124292181581  
 89,M\_h2o\_c\_exchange\_reverse::M\_h2o\_c --> M\_h2o\_e,7.094141911569084  
 90,M\_pi\_c\_exchange::M\_pi\_e --> M\_pi\_c,0.8245640240851799  
 92,M\_nh3\_c\_exchange::M\_nh3\_e --> M\_nh3\_c,1.2222205427275048  
 109,M\_bio\_exchange::M\_bio\_c --> M\_bio\_e,0.224145492724381

## Steady State Fluxes in Anaerobic Environment

1,R\_glk\_hex::M\_pep\_c+M\_glc\_D\_e --> M\_pyr\_c+M\_g6p\_c,2.5  
 3,R\_pgi::M\_g6p\_c --> M\_f6p\_c,1.7619160196720376  
 5,R\_pfk::M\_atp\_c+M\_f6p\_c --> M\_adp\_c+M\_fdp\_c,2.1760804023199034  
 7,R\_fbaA::M\_fdp\_c --> M\_dhap\_c+M\_g3p\_c,2.1760804023199034  
 9,R\_tpiA::M\_dhap\_c --> M\_g3p\_c,2.1760804023199034  
 11,R\_gapA::M\_g3p\_c+M\_nad\_c+M\_pi\_c --> M\_13dpg\_c+M\_h\_c+M\_nadh\_c,4.536200074721678  
 13,R\_pgk::M\_13dpg\_c+M\_adp\_c --> M\_3pg\_c+M\_atp\_c,4.536200074721678  
 15,R\_gpm::M\_3pg\_c --> M\_2pg\_c,4.410412042690574  
 17,R\_eno::M\_2pg\_c --> M\_h2o\_c+M\_pep\_c,4.410412042690574  
 19,R\_pyk::M\_adp\_c+M\_pep\_c --> M\_atp\_c+M\_pyr\_c,1.6258166202202018  
 21,R\_ppc::M\_co2\_c+M\_h2o\_c+M\_pep\_c --> M\_oaa\_c+M\_pi\_c,0.24094798435048903  
 24,R\_zwf::M\_g6p\_c+M\_nadp\_c --> M\_6pgl\_c+M\_h\_c+M\_nadph\_c,0.7208469839600637  
 26,R\_pgl::M\_6pgl\_c+M\_h2o\_c --> M\_6pgc\_c,0.7208469839600637  
 27,R\_gnd::M\_6pgc\_c+M\_nadp\_c --> M\_co2\_c+M\_nadph\_c+M\_ru5p\_D\_c+M\_h\_c,0.7208469839600637  
 28,R\_rpe::M\_ru5p\_D\_c --> M\_xu5p\_D\_c,0.4201258609038855  
 31,R\_rpi\_reverse::M\_ru5p\_D\_c --> M\_r5p\_c,0.3007211230561782  
 32,R\_talAB::M\_g3p\_c+M\_s7p\_c --> M\_e4p\_c+M\_f6p\_c,0.22523989554660467  
 34,R\_tkt1::M\_r5p\_c+M\_xu5p\_D\_c --> M\_g3p\_c+M\_s7p\_c,0.22523989554660467  
 36,R\_tkt2::M\_e4p\_c+M\_xu5p\_D\_c --> M\_f6p\_c+M\_g3p\_c,0.1948859653572808  
 40,R\_gltA::M\_accoa\_c+M\_h2o\_c+M\_oaa\_c --> M\_cit\_c+M\_coa\_c,0.09071705064061365  
 41,R\_acn::M\_cit\_c --> M\_icit\_c,0.09071705064061365  
 43,R\_icd::M\_icit\_c+M\_nadp\_c --> M\_akg\_c+M\_co2\_c+M\_nadph\_c+M\_h\_c,0.09071705064061365  
 53,R\_gdhA::M\_akg\_c+M\_nadph\_c+M\_nh3\_c+M\_h\_c --> M\_glu\_L\_c+M\_h2o\_c+M\_nadp\_c,0.4369872869444186  
 55,R\_glnA::M\_glu\_L\_c+M\_atp\_c+M\_nh3\_c --> M\_gln\_L\_c+M\_adp\_c+M\_pi\_c,0.02149999985986181  
 71,R\_pta::M\_accoa\_c+M\_pi\_c --> M\_actp\_c+M\_coa\_c,1.0645625012086923  
 73,R\_ackA::M\_actp\_c+M\_adp\_c --> M\_ac\_c+M\_atp\_c,1.0645625012086923  
 76,R\_adhE::M\_accoa\_c+2.0\*M\_h\_c+2.0\*M\_nadh\_c --> M\_coa\_c+M\_etoh\_c+2.0\*M\_nad\_c,2.4172210766704394  
 80,R\_pflAB::M\_coa\_c+M\_pyr\_c --> M\_accoa\_c+M\_for\_c,3.8876265552885765  
 83,R\_Biomass\_Ecoli\_core\_w\_GAM::1.496\*M\_3pg\_c+3.7478\*M\_accoa\_c+59.81\*M\_atp\_c+0.361\*M\_e4p\_c+0.0709\*M\_f6p\_c+0.129\*M\_g3p\_c  
 +0.205\*M\_g6p\_c+0.2557\*M\_gln\_L\_c+4.9414\*M\_glu\_L\_c+59.81\*M\_h2o\_c+3.547\*M\_nad\_c+13.0279\*M\_nadph\_c+1.7867\*M\_oaa\_c+0.5191\*M\_pep\_c  
 +2.8328\*M\_pyr\_c+0.8977\*M\_r5p\_c  
 --> 59.81\*M\_adp\_c+4.1182\*M\_akg\_c+3.7478\*M\_coa\_c+59.81\*M\_h\_c+3.547\*M\_nadh\_c+13.0279\*M\_nadp\_c+59.81\*M\_pi\_c+M\_bio\_c,0.08408290911170048  
 85,M\_co2\_c\_exchange::M\_co2\_c --> M\_co2\_e,0.5706160502501884

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86,M_h_c_exchange::M_h_c --> M_h_e,5.8261804469679275
88,M_h2o_c_exchange::M_h2o_e --> M_h2o_c,1.2341114832869793
90,M_pi_c_exchange::M_pi_e --> M_pi_c,0.30931579774921364
92,M_nh3_c_exchange::M_nh3_e --> M_nh3_c,0.45848728680428036
98,M_ac_c_exchange::M_ac_c --> M_ac_e,1.0645625012086923
105,M_etoh_c_exchange::M_etoh_c --> M_etoh_e,2.4172210766704394
107,M_for_c_exchange::M_for_c --> M_for_e,3.8876265552885765
109,M_bio_exchange::M_bio_c --> M_bio_e,0.08408290911170048

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